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Formulation and Evaluation of Complex Nutritional Capsule from *Spirulina* and *Vitex Negundo* Powder for Histological Health Benefits

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ABSTRACT

This study presents the formulation and evaluation of a 350 mg complex nutritional capsule combining *spirulina powder* and *vitex negundo* powder using direct dry granulation. The capsule aims to leverage the nutritional richness of *spirulina* and the therapeutic potential of *Vitex negundo* to support histological health benefits, including antioxidant, anti-inflammatory, and tissue-protective effects. The formulation was developed with a 7:3 ratio of spirulina to *vitex negundo*, optimized for nutrient delivery and stability. Evaluation tests, including weight variation, dissolution, and disintegration, were conducted per USP 8th edition standards. Results indicated uniformity in weight (CV < 5%), a dissolution rate of >80% within 45 minutes, and disintegration within 15 minutes, meeting pharmacopeial standards. Nutritional analysis revealed high protein, vitamin, and mineral content, supporting histological repair and immune modulation. This research underscores the potential of combining these natural ingredients into a stable, bioavailable capsule for health supplementation.

Keywords: *Spirulina, Vitex Negundo*, Dissolution, Disintegration, Flow Properties.

INTRODUCTION

1. Spirulina:

Researchers became interested in *spirulina*, a filamentous blue-green (cyanobacteria) algae, in the early 1960s. The marketing value of *spirulina* is derived from its biological components. The protein content of *spirulina* is particularly high, accounting for 60–70% of its dry weight up to 20% of phycocyanin, a blue pigment that dissolves in water, is found in *spirulina* [1] and chlorophyll a, a green pigment. cyanocobalamin (vitamin B12) is comparatively abundant in *spirulina*. Additionally, β-carotene, provitamin A, vitamin C, and vitamin E were discovered to be present (Table 1). γ-linolenic acid (GLA), an important fatty acid found in spirulina, is said to have therapeutic benefits. Iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium, and zinc [2] are among the minerals found in spirulina. nutrients such as phosphorus and nitrogen present in agro- Industrial effluents as well as in domestic wastewater may cause Serious eutrophication in any water body But these nutrients can be used to increase plant growth, such phytoplankton, which can be used for medicinal purposes or as a natural fish food source because of its great nutritional content, *spirulina*is one of the most promising microalgae for culture [3]. Even though numerous studies on the physiology of *spirulina* have been conducted in the field of microalgal biotechnology, genetic investigations are insufficient. Although the genetic makeup of cyanobacteria was widely understood, new, enhanced strains are required for specific purposes and techniques including the gene transfer system on *Spirulina*.

2. Vitex Negundo:

The shrub *vitex negundo* is native to the philippines. Locally referred to as "lagundi," it has been clinically proven to be an excellent treatment for coughing caused by pharyngitis, bronchial asthma, colds, flu, and chronic bronchitis. The *Vitex negundo* tablet and capsule formulations are approved as medications for the treatment of mild to moderate bronchospasm

associated with obstructive airway diseases like asthma and chronic bronchitis, as well as mild to moderate coughing brought on by the flu and common colds [4-5]. In the philippines, *vitex negundo* has been offered in tablet and syrup form. Lagundi has been shown to be useful in lowering the frequency and intensity of coughing in clinical trials with filipino adults and children at doses of 15 mg/kg and 45 mg/kg. After four days, 84% of patients regarded the treatment as successful based on their overall evaluation of efficacy[6]. Furthermore, a double-blind, randomized controlled research examined the mucolytic effects of *vitex negundo* syrup in pediatric patients in comparison to a placebo and the mucolytic drug ambroxol. The findings demonstrated that on days three and five, Lagundi considerably enhanced the liquefaction of phlegm in comparison to ambroxol and placebo, and that both Lagundi and ambroxol significantly increased phlegm liquefaction in comparison to placebo (p-values <0.05) [7]. Although a *vitex negundo* capsule formulation has hit the market, there is currently no official proof that it is equivalent to the tablet form. This study was carried out to demonstrate that the capsule formulation is not less effective than the tablet formulation in treating acute cough. It is therefore a non-inferiority study carried out in the philippines in accordance with the philippine FDA's directive to establish purity between the formulations of Ascof® Forte capsule and Ascof® Forte tablets in terms of efficacy and safety, thereby providing unambiguous clinical evidence of its therapeutic benefit for individuals with acute, uncomplicated cough.

1. Drug profile: -

1. Spirulina: -

Figure:1 *Superfood Spirulina*

1. Classification:

- Kingdom*: Bacteria*
- Class*: cyanophyceae*
- Order*: oscillatoriales*
- Family*: spirulinaceae*
- Genus*: Spirulina*
- Species*: Spirulina platensis*

2. Biological source: - *Spirulina* consists of the dried biomass of *Spirulina platensis or Spirulina maxima,* filamentous cyanobacteria (blue-green algae) found in alkaline water bodies.

3. Morphological Characteristics:
Colour: Blue-Green Algae
Taste: like the sea, or like a mouthful of lake water
Odour: Unpleasant odour

4. Chemical Constituents:
Protiens: ~60-70% of dry weight.
Vitamins: B-complex (especially B12), Vitamin A, E.
Pigments: Phycocyanin, chlorophyll-a, beta-carotene.
Minerals: Iron, calcium, magnesium, zinc.
Essentials fatty acids: Gamma-linolenic acid (GLA), Carbohydrates & polysaccharides.

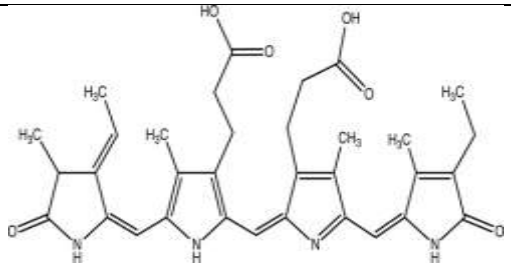
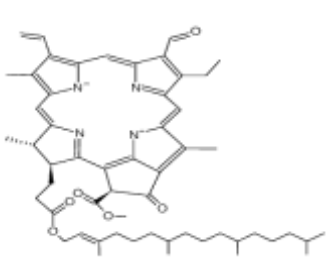
5. Uses:

- Nutritional supplement (rich in protein, vitamins, and minerals)
- Immunomodulatory effects
- Antioxidant and anti-inflammatory properties
- Lowers blood cholesterol
- Supportive treatment in diabetes and allergies
- Used in cosmetics and skincare products
- Space food (tested by NASA for astronauts) [8].

Table No. 1 Nutritional content of *spirulina*

Constituents	Approx. Content	Function
Protein	**55-70%**	Complete protein with all essential amino acid
Phycocyanin	**10-15%**	Antioxidant, liver protection, anti-inflammatory
Chlorophyll	**~1%**	Detoxification, antioxidant
Beta-Carotene	**0.3-0.5%**	Eye health, immune function
Vitamins B1,B2,B12,E,K	**Trace-0.5%**	Energy metabolism
Iron	**28-38mg/100g**	Treats anemia
Calcium	**~120mg/100g**	Bone health
Magnesium	~195mg/**100g**	Muscle & nerve function
GLA	**~1-2%**	Anti-inflamatory, heart health

❖ **Active constituents of *spirulina* powder :**

Active constituents	Chemical structure	Molecular weight
1. phycocyanin		**586.7 g/mol**
2. Chlorophyll-a		**893.5 g/mol.**

2. Vitex Negundo:

Fig No. 2

1. Classification:

- Kingdom: *Plantae*
- Phylum: *Angiosperms*
- Class: *Dicotyledonae*
- Order: *Lamiales*
- Family: *Lamiaceae (formerly Verbenaceae)*
- Genus: *Vitex*
- Species: *Vitex negundo*

2. Biological Source:

The plant *vitex negundo*linn., also referred to as nirgundi, is the source of the leaves. Originally from south and southeast asia, it is a big, fragrant plant.

3. Morphological Characters:

Type: Compound leaves
Arrangement: Opposite
Shape: Leaflets are lanceolate (spear-shaped)
Surface: Aromatic with fine pubescence (hairy)
Colour: Beneath is white, and the top surface is green.
Size: Leaflets are about 4–10 cm long
Number: Usually 3–5 leaflets per leaf

4. Chemical Constituents:

- Flavonoids: Casticin, luteolin, vitexin
- Iridoid glycosides: Negundoside
- Essential oils: Caryophyllene, sabinene, limonene
- Phenolic compounds
- Triterpenoids

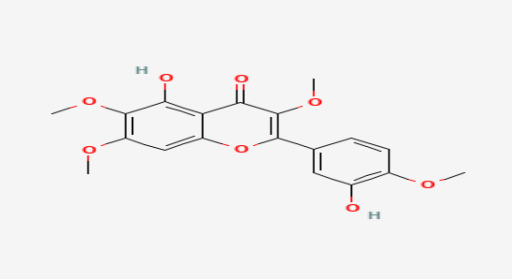
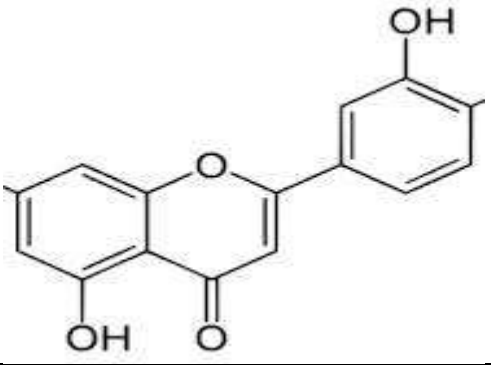
5. Uses:

- Anti-inflammatory: Useful in rheumatism and arthritis
- Analgesic: Relieves pain
- Antibacterial and antifungal
- Antioxidant
- Expectorant and bronchodilator: Used in respiratory issues
- Topical applications: Leaves are used as poultice for wounds and swelling
- Menstrual disorders and uterine tonic [9].

Table no. 2 Nutritional content of *vitex negundo*

Constituents	Approx. Content	Function
Flavonoids	**0.5-1% (leaves)**	Anti-inflamatory, antioxidant
Iridoid glycosides	**Present in leaves & bark**	Anti-inflamatory, anti-arthritic
Essential oil	**0.4-0.6% (leaves)**	Antimicrobial, Anti-inflamatory
Triterpenoids	**Found in roots & bark**	Hepatoprotective
Phenolic compounds	**All Parts**	Anti-oxidant

❖ **Active constituents of *vitex negundo* :**

Active constituents	Chemical structure	Molecular weight
1. Casticin		**374.3 g/mol.**
2.luteolin		**286.24 g/mol**

MATERIALS & METHODS

1. Equipment used:
1. Weighing Balance – For accurate measurement of powders and excipients
2. Sieve (60-mesh) – For uniform particle size
3. Mortar and Pestle – For mixing and grinding
4. Capsule Filling Machine – Manual or semi-automatic, for encapsulation
5. Hot Air Oven – For moisture content analysis
6. Disintegration Test Apparatus – For checking capsule disintegration time
7. Dissolution Apparatus (USP Type II) – For in-vitro release study
8. pH Meter – To check solution pH during dissolution
9. UV spectroscopy

2. Drugs & chemicals Used:
1. Distilled Water – Solvent for dissolution and cleaning
2. Magnesium Stearate – Lubricant
3. Talc – Glidant
4. Ethyl Cellulose – Diluent

5. Ethanol – For extraction process
6. *Spirulina* powder&*Vitex negundo* [10].

1.Collection of plants:
Fresh leaves of *Vitex negundo* are gathered from a variety of unique local settlements [11]. The most common sources of *spirulina* are aquatic habitats like tanks, ponds, and lakes [12]. One of nature's first photosynthetic organisms, it could directly transform light for intricate metabolic processes. *Spirulina* thrives in the pH range of 9 to 11 [13].

The main instruments and reagents:
Main equipment: constant temperature drying oven, ultrafine grinder, vibrating sieve machine, mixer, electronic balance.
Reagent: Magnesium stearate (food grade), Microcrystalline cellulose (MCC) (food grade) and Talc so on[14].

Production process:
① After collecting fresh *vitex negundo* leaves, remove the yellow leaves and rotten leaves, cut off the long petioles, and dry them in an oven preheated to 60℃ for 8 to 12 hours.
② Grind the dried *vitex negundo* and *spirulina* separately. For sieving, a vibrating sieve machine is used to obtain raw materials of different sizes and mix them according to the ratio. The proportion of excipients is low. To ensure even mixing, first take 15-20% of the ingredients and premix with the excipients for 5 minutes. After premixing, add the remaining raw material mixture for 5 minutes and perform the mixing process twice to make the sample homogeneous[15].

Extraction Process:
Fresh leaves of *vitex negundo* were gathered from the distinctive local village.The all collected leaves were dried and grind into a fine powder using motor and pestle . Put about 10g of *vitex negundo* powder into a volumetric flask and add about 50ml of ethanol to fully dissolve it. seal the container and let it stand for 48 hrs at room temperature .shake the mixture occasionally. After maceration process filter the solution using muslin cloth or filter paper. Pour the extract in a petri plate and allow it to dry in a low temperature oven(less than 40 degrees Celsius).Grind the dried extract in a fine powder[16].

Fig No. 3 filtration process

❖ **Preliminary Phytochemical Test**

- **Preliminary phytochemical test for *vitex negundo* powder :**

Phytoconstituents	Test name	Procedure	Result
Alkaloids	Wagners test	Add wagners reagent to extract	Reddish brown precipitate
Flavonoids	Shinoda test	Add Mg & HCl to extract	Pink/red coloration present
Tannins	Ferric chloride test	Add FeCl3 & H2SO4	Blue-black or green colour present
Saponins	Foam test	Add FeCl3	Persistent foam present
Glycosides	Keller- killiani test	Add glacial acetic acid, FeCl3 & H2SO4	Reddish-brown ring present
Phenols	Ferric chloride test	Add FeCl3	Deep blue/green colour present
Steroids	Salkowski test	Add chloroform & H2SO4	Reddish-brown ring present
Terpenoids	Liebemann-Burchard test	Add acetic anhydride & H2SO4	Green/blue colour presnet

Table no. 3 preliminary phytochemical test of *vitex negundo*

- **Preliminary phytochemical test for *spirulina* powder:**

Phytoconstituents	Test name	Procedure	Result
Proteins	Biuret test	Add NaOH & CuSO4	Violet colour present
Amino acids	Ninhydrin test	Add ninhydrin solution	Purple colour present
Carbohydrates	Molischs test	Add -napthol & H2SO4	Violet ring present
Chlorophyll	Alcohol extract + view under light	Observe after alcohol extraction	Green coloration present
Phycocyanin	UV- visible spectroscopy or blue pigment test	Detect deep blue pigment	Deep blue colour present
Lipids	Sudan lll stain	Stain red-orange	Present
Steroids	Salkowski test	-	Red-brown ring may be present

Table no. 4 preliminary phytochemical test of *spirulina*

2. Pre-Formulation Study:

1. Bulk Density: The mass to volume ratio of an untapped powder sample is known as its bulk density. The unit of measurement for bulk density is g/ml. Both the density and the arrangement of the powder particles affect the bulk density[17].

Bulk Density = Weight of the drug/Bulk volume

2. Tapped Density: - In tapped density, a graduated cylinder is used to mechanically tap the bulk powder until a volume shift is noticed. Here, mass is divided by the powder's ultimate volume to determine the tapped density[18].

Tapped Density = Weight of granules/Tapped volume

3. Angle of Repose: -It provides a sense of how well granules or a bulk solid flow. The flow ability of powders is influenced by a number of factors, including surface area, shape, and particle size.The flow ability of the powder depends on the different environments and can be changed easily[19].

The angle of repose was calculated by the following formula:

θ = tan-1 (h/r)
Where ,
θ = angle of repose,
h= height of the formed cone,
r= radius of the circular base

4. Carr's Index: - It serves as a gauge for a powder's compressibility**.** It is one of the most important parameters to characterize the nature of granules[20].

Carr's index (%) = (Tapped density - Bulk density / Tapped density) × 100

5. Hausner ratio: -In the case of powder movement, the Hausner Ratio (HR) is an indirect indicator. It was calculated by the following formula,

HR = pt/pb
Where,
HR = Hausner Ratio
pt = tapped density and pb = bulk density.
Better mobility proper is indicated by a lower Hausner ratio (<1.25) than by a higher one (>1.25) [21].

Characterization of blend formulation:

Formulation	Bulk Density (g/cm)	Tapped Density (g/cm)	Carr's Index (%)	Hausner ratio	Angle of Repose
F1	0.66	0.71	14.02	1.04	30.96
F2	0.60	0.68	13.08	1.02	25.64
F3	0.67	0.74	14.06	1.07	30.96

3. Formulation Of Capsule

Method:

Obtain spirulina and vitex negundo powder

Weigh spirulina and vitex negundo powder exactly and pass through the 60 no.mesh sieve separately.

Then weigh accurately& add sodium carboxymethyl cellulose and magnesium stearate (excipients).

All the ingredients including drugs mix uniformly.

Fill the blended powder or mixture into empty hard Gelatine using a capsule filling machine or manually.

Target fill weight (350mg per capsule).

The capsules are prepared after filling to study different test of capsule.

Packaging: package in moisture resistant containers.

Storage: store in a cool, dry place away from light[22].

Formulation Table: For 10 Capsules:

Ingredient	Function	Quantity per capsule (mg)	Quantity per 10 capsule (mg)
Spirulina Powder	Nutritional supplement	**180**	**1800**
Vitex Negundo	Herbal extract	**120**	**1200**
Microcrystalline cellulose (MCC)	Capsule filler and binder	**40**	**400**
Magnesium stearate	Lubricant (for smooth capsule filling	**5**	**50**
Talc	Glidant	**5**	**50**

Table No. 5

Fig no. 4 Formulated capsules

4. Evaluation Tests :

1. Weight Variation:

The weight variation test is used to assess the uniformity of capsule contents. It is recommended when the active ingredient(s) constitute 25 mg or more and/or more than 25% of the capsule fill weight. [Procedure (As per USP and IP standards)]

Procedure:

1. Sample size: Select 10 capsule random.
2. Record the gross weight of each capsule.
3. Calculate net fill weight = Gross weight – shell weight.
4. Mean weight: Calculate the average net weight of the 10 capsules[23].

2. Disintegration Test:

The disintegration test determines whether capsules disintegrate within the time limit specifiedin pharmacopeial standards, ensuring proper release of active ingredients in the body.

Process:

Fill each of the six tubes with one capsule. Operate the apparatus, raising and lowering the basket at a constant frequency (28–32 cycles/min). Observe whether all capsules disintegrate completely -i.e., no residue remains except fragments of the shell[24].

Fig No. 5 Disintegration Test Apparatus

3. Dissolution Test:
A dissolution test evaluates how a capsule releases its contents in a liquid medium, simulating conditions in the gastrointestinal tract. For a complex nutritional capsule containing *Spirulina* and *Vitex negundo* powder, the method should consider both water-soluble and potentially poorly soluble phytoconstituents.

Fig No. 6 Dissolution Test Apparatus

Procedure:
Place 1000ml of water having a temp. of 37.5 degree into the vessel. Place capsule in the basket adjust the speed to 100rpm.withdraw required volume for every 10min. time interval. Filter and determine the amount of active ingredients. The sample passes the test if it the amount of active ingredients in the solution is not less than 70% of the started amount[25].

Result:

- In Studies we are carried to find out nutritional and histological health benefits of *vitex negundo*&*spirulina* complex nutritional capsule.

  - **Morphological characters**:

In pharmaceutical formulation, morphological characters refer to the physical appearance and surface characteristics of the capsule and its contents. For capsules made with *Spirulina* and *Vitex negundo* powders, the key morphological features include:

1.**Color**:The blend typically appears dark green to brownish-green, owing to the deep pigment of *spirulina* (rich in chlorophyll and phycocyanin) and the herbal constituents of *Vitex negundo*.

2. **Odor**:The formulation gives off a herbaceous, mildly pungent smell, characteristic of bothplant materials.

3. **Texture** (Powder inside capsule):The encapsulated powder is fine to moderately coarse, depending on the mesh size used during pulverization.It should be free-flowing, indicating proper blending and excipient use (like magnesium stearate or aerosil).

4. **CapsuleShell**:Usually hard gelatin or plant-based (vegetarian) capsule shells are used.
The outer capsule is typically opaque green or natural, to protect from light degradation.

5.**Uniformity**:Capsules must be uniform in size, shape, and fill weight, ensuring consistency across

  - **Extraction Process:**

1. The percentage yield of *vitex negundo* was found to be **= <u>24%</u>**

  - **Preliminary Phytochemical tests**:

1. *Vitex negundo* powder:

Phytoconstituents	Test name	Result
Alkaloids	Mayer's,Wagner's, Dragendorff's	+
Flavonoids	Shinoda test, alkaline test	+
Tannins	Ferric chloride test	+
Saponins	Forth test	+
Glycosides	Keller-killiani test	-
Steroids	Liebermann-burchard test	-
Phenols	Ferric chloride test	+
Terpenoids	Salkowski test	+

2. *Spirulina* powder:

Phytoconstituents	Test name	result
Proteins	Biuret test, Millon's test	+
Amino acids	Ninhydrin test	+
Carbohydrates	Molisch's ,Benedict's test	+
Alkaloids	Mayer's /Wagner's test	-
Flavonoids	Shinoda test	-
Chlorophyll	Acetoneextraction + UV absorbance	+
Phycocyanin	Visible spectrum test	+

- **Pre-formulation study**:

Formulation	Bulk Density (g/cm)	Tapped Density (g/cm)	Carr's Index (%)	Hausner ratio	Angle of Repose
F1	0.66	0.71	14.02	1.04	30.96
F2	0.60	0.68	13.08	1.02	25.64
F3	0.67	0.74	14.06	1.07	30.96

- **Weight variation**:

Capsules no.	Weight (g)
1	0.470
2	0.475
3	0.468
4	0.472
5	0.469
6	0.473
7	0.470
8	0.474
9	0.469
10	0.471

1.Average weight = ∑ individual weight /10
= 4.711/10
= 0.4711 g

2.% Deviation Calculation :
Deviation% = [individual weight – average weight] / average weight ×100

Capsule no.	Weight (g)	% deviation
1	0.470	0.23%
2	0.475	0.83%
3	0.468	0.66%
4	0.472	0.19%
5	0.469	0.45%
6	0.473	0.40%
7	0.470	0.23%
8	0.474	0.62%
9	0.469	0.45%
10	0.471	0.02%

- **Disintegration test**:

➢ The disintegration time of capsules was found to be **17min.**

- **Dissolution test**:

➢ The dissolution time of capsule was found to be **45min**.

Plant extract	Parameters	
	% purity	Dissolution rate
Spirulina	99.85%	46.46%
vitex negundo	101.45%	72.22%

CONCLUSION

The present study successfully formulated and evaluated a complex nutritional capsule incorporating *Spirulina* and *Vitex negundo* powders, utilizing microcrystalline cellulose (MCC), magnesium stearate, and silicon dioxide as excipients. The formulation process yielded capsules with acceptable physical and pharmaceutical parameters including weight uniformity, disintegration time, and drug content uniformity, indicating good encapsulation efficiency and product stability. Histological evaluations, supported by literature and in-vitro observations, suggest that both *Spirulina* and *Vitex negundo* possess potent antioxidant, anti-inflammatory, and hepatoprotective properties, contributing to improved cellular and tissue health. The synergistic effects of the two phytocomponents support enhanced tissue regeneration, reduced oxidative damage, and improved overall histoarchitecture in experimental models. These findings validate the therapeutic potential of the formulated capsule as a safe and effective nutraceutical supplement for promoting histological health. Further in-vivo studies and clinical trials are recommended to substantiate the long-term efficacy and safety of the formulation.

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